Effect of normabaric hyperoxia treatment on neuronal damage following fluid percussion injury in the striatum of mice: A morphological approach

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Traumatic brain injury (TBI) causes significant mortality in most developing countries worldwide. At present, it is imperative to identify a treatment to address the devastating post-TBI consequences. Therefore, the present study has been performed to assess the specific effect of immediate exposure to normabaric hyperoxia (NBO) after fluid percussion injury (FPI) in the striatum of mice. To execute FPI, mice were anesthetised and sorted into (i) a TBI group, (ii) a sham group without injury and (iii) a TBI group treated with immediate exposure to NBO for 3 h. Afterwards, brains were harvested for morphological assessment. The results revealed no changes in morphological and neuronal damage in the sham group as compared to the TBI group. Conversely, the TBI group showed severe morphological changes as well as neuronal damage as compared to the TBI group exposed to NBO for 3 h. Interestingly, our findings also suggested that NBO treatment could diminish the neuronal damage in the striatum of mice after FPI. Neuronal damage was evaluated at different points of injury and the neighbouring areas using morphology, neuronal apoptotic cell death and pan-neuronal markers to determine the complete neuronal structure. In conclusion, immediate exposure to NBO following FPI could be a potential therapeutic approach to reduce neuronal damage in the TBI model.

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1. Introduction

Traumatic brain injury (TBI) leads to significant morbidity and mortality in most developing countries worldwide. TBI can be caused by either closed or open head injuries. Closed head injuries are more harmful to the brain due to the build-up of pressure (intracranial pressure (ICP)) within the unbroken cranium that slows the cerebral blood flow (CBF) and causes low brain oxygenation in the damaged region. As a result, TBI victims suffer diverse cognitive impairments and motor disabilities depending on the severity of damage and the site of injury in the brain. Fluid percussion injury (FPI) is an extensively used experimental model used to generate reproducible closed brain injuries in rat models. FPI involves an impact that leads to brain trauma and is performed by injecting a high-pressure fluid column into the brain tissue. However, the majority of these studies focused on cortical lesions, although tissue damage might extend to deep cerebral areas such as the basal ganglia. A number of research groups have attempted to develop novel and innovative protocols to replicate closed traumatic brain injuries in animal models, but very few experimental models have been successful (Haruddin 2010; Muthuraju *et al.* 2012). Very few TBI patients were reported to exhibit focal lesions in basal ganglia, but chronic demolition in basal ganglia is thought to impair motor coordination and long-term memory acquisition (El Massioui *et al.* 2007).

The striatum is one of the main structures in the brain. Major inputs to basal ganglia originate from the

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striatum, with medium spiny neurons constituting approximately 95% of its neuronal types (Voulalas *et al.* 2005). Striatal medium spiny neurons play an important role in the motor control and long-term memory acquisition (El Massioui *et al.* 2007).

In closed head TBI, the primary neuronal damage occurs at the time of collision, while the secondary neuronal damage occurs hours or days after the initial events. Primary damage includes the breakdown of the blood–brain barrier (BBB) and physical tissue damage, followed by morphological, biochemical and molecular changes. To date, the most common strategy for TBI management includes monitoring the intracranial pressure and treatment to reduce ICP immediately after a TBI insult. It is believed that secondary neuronal damage might be controlled by reducing ICP and increasing CBF following TBI using a therapeutic approach; however, such an approach has not been established. One type of potential therapeutic intervention to reduce ICP, increase CBF and improve brain oxygenation might be immediate exposure to NBO after a TBI insult. NBO treatment involves the administration of 100% oxygen in the first few hours after a TBI insult. Therefore, in the present study, we hypothesised that immediate exposure to normabaric hyperoxia for 3 h could be a possible therapeutic approach for minimizing the secondary neuronal damage of TBI in a mouse model.

2. Material and methods

2.1 *Surgical preparation and fluid percussion injury*

All animal handling procedures followed were in accordance with the Guide for the Care and Use of Laboratory Animals prescribed by the National Institutes of Health and were approved by the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia. Adult male C57BL/J6 mice weighing between 25 and 30 g were surgically prepared under Ketamine+Xylazine (2.6/0.16 mg/kg of body weight, respectively) (Carbonell *et al.* 1998). The animals were placed in a stereotactic frame, and a syringe hub was placed 0.2 mm anterior and 2.3 mm lateral to the bregma. Dental acrylic was then poured around the syringe hub, and the animals were returned to their cages.

Twenty-four hours after the surgical procedure, the mice were again anesthetised under intra-peritoneal Ketamine+Xylazine (2.6/0.16 mg/kg of body weight, respectively) administration. Then, the mice were divided into three groups: (i) a sham-injury (control) group (n=18), in which the mice were anesthetised and connected to the injury device but the pendulum was not released; (ii) a TBI group (n=18) with injury but without exposure to NBO; and (iii) a TBI group (n=18) with injury and immediate exposure to NBO for 3 h. Each group consisted of 6 animals and were used to study the morphological changes, apoptotic neuronal damage and pan-neuronal markers to assess the soma, axons and spine morphologies at 3 h post injury.

2.2 *Induction of FPI injury*

TBI was induced in the mice using a fluid percussion device (FPD) (Dragonfly Inc., Model HPD 1700, Virginia, and USA) with a modified method (Carbonell *et al.* 1998; Ling *et al.* 2004). The mice were connected to the FPI device with saline-filled high-pressure tubing. A pulse of 21–23 ms at 3.6±0.1 atm (Carbonell *et al.* 1998) was rapidly injected into the cranial cavity. The strike from the pendulum was calibrated using a transducer connected to a charge amplifier and a digital oscilloscope for data acquisition. The TBI-induced mice were then immediately exposed to NBO treatment for 3 h. The untreated experimental group was kept in normal conditions for 3 h. After 3 h of exposure to NBO, the animals were sacrificed for further experimentation. The sham-operated animals were surgically prepared but were not subjected to FPI.

2.3 *Oxygen therapy*

Pure oxygen was delivered immediately after FPI to six animals in each group. The mice were placed in a closed chamber to receive 97±3% O_2 from an oxygen tank for 3 h (Tolias *et al.* 2004) under normal atmospheric pressure.

2.4 *Tissue processing*

The animals were anaesthetised with Ketamine+Xylazine (2.6/0.16 mg/kg of body weight, respectively) and were immediately perfused transcardially using ice-cold phosphate-buffered saline (0.1 M, pH 7.4) followed by fixation using ice-cold 4% para-formaldehyde (dissolved in 0.1 M PBS, pH 7.4). Then, the brain was removed from the cranium and fixed in the same conditions for 24 h at room temperature.

2.4.1 *Morphological changes:* The brains from each group of mice were dehydrated and embedded in paraffin wax. Coronal sections of approximately 5 μm were made. The sections were deparaffinised and rehydrated with xylene for 10 min, 5 min 100% ethanol, 5 min 95% ethanol, 5 min 80% ethanol, and 5 min deionised H_2O. The sections were stained using the following haematoxylin staining procedure: stain in haematoxylin (20 min), rinse with deionised water, rinse

with 5 min tap water (to allow the stain to develop), dip 8 times (rapidly) in acidic ethanol (to destain), rinse with 2 min tap water, and rinse with 2 min deionised water. The eosin staining and dehydration protocol is as follows: 5 min 95% ethanol, 5 min 100% ethanol (blotting excess ethanol before washing with xylene), and 5 min xylene. Cover slip slides were prepared using DPX in xylene. A drop of DPX was placed on the slide using a glass rod, taking care to leave no bubbles. The cover slip was held at an angle and allowed to fall gently onto the slide. The DPX was allowed to spread beneath the cover slip so that the entire section was covered. Then, the sections were dried overnight in the hood. Light microscopy was used to evaluate the morphology of the right striatum of both the control group and the experimental group with hyperoxia treatment. The small, dense, irregularly shaped pyknotic cells were considered to be dead. Photographs were captured via a compound light microscope attached to a CCD camera. The images were uploaded to Adobe Photoshop 7.0 with a resolution of 300 dpi and the brightness and contrast were corrected [modified Chopp *et al.* (1991) method].

2.5 *Apoptotic neuronal cell death assay*

2.5.1 *Detection by fluorescence microscopy:* The sections from six brains in each group were deparaffinised and rehydrated using the following protocol: 10 min xylene, 5 min 100% ethanol, 5 min 95% ethanol, 5 min 80% ethano, and 5 min deionised H_2O. Then, the sections were washed with ice-cold phosphate-buffered saline (0.1 M, pH 7.4) 3 times for 5 min. After washing, the sections were incubated with 5% Triton X for 30 min and then washed with PBS 3 times each for 5 min. Annexin V-FITC was added, and the sections were incubated for 1 h in a dark place to check for a reaction. The sections were observed under a fluorescence microscope using a dual filter set for FITC. The cells that had bound Annexin V-FITC showed green staining in the plasma membrane, whereas the cells that had lost membrane integrity showed red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane). The number of intact cells per defined area was determined, and the number of dead cells was counted at 40× magnification [modified Xu *et al.* (2010) method].

2.5.2 *Neuronal structure using immunofluorescence:* The sections from each group were deparaffinised and rehydrated using the following protocol: 10 min xylene, 5 min 100% ethanol, 5 min 95% ethanol, 5 min 80% ethanol, and 5 min deionised H_2O. Then, the sections were washed with ice-cold phosphate-buffered saline (0.1 M, pH 7.4) 3 times for 5 min. The sections were blocked with blocking buffer (1% BSA, 5% serum, 0.2% Triton X in PBS) for 1 hr at RT. Afterwards, the sections were washed 3 times for 5 min and incubated with Milli-Mark fluoro pan-neuronal marker (1:50) for 2 h at RT (dilute antibody in blocking buffer). The sections were then washed with PBS 5 min and mounted on slides using a cover slip with light diagnostics mounting fluid. The slides were viewed using a fluorescent microscope with an FITC filter at 100× magnification.

2.6 *Statistical analysis*

The data are expressed as the mean±standard error of the mean (SEM). Statistical analyses were performed using Prism 5 software (GraphPad, San Diego, CA) and SigmaPlot. The means of the three groups were compared with a one-way non-parametric Kruskal-Wallis ANOVA; the comparisons of the means from multiple groups with one control were analysed with a one-way ANOVA and Dunnet's *post hoc* test. (*$p<0.05$ compared to sham and FPI) (figure 1).

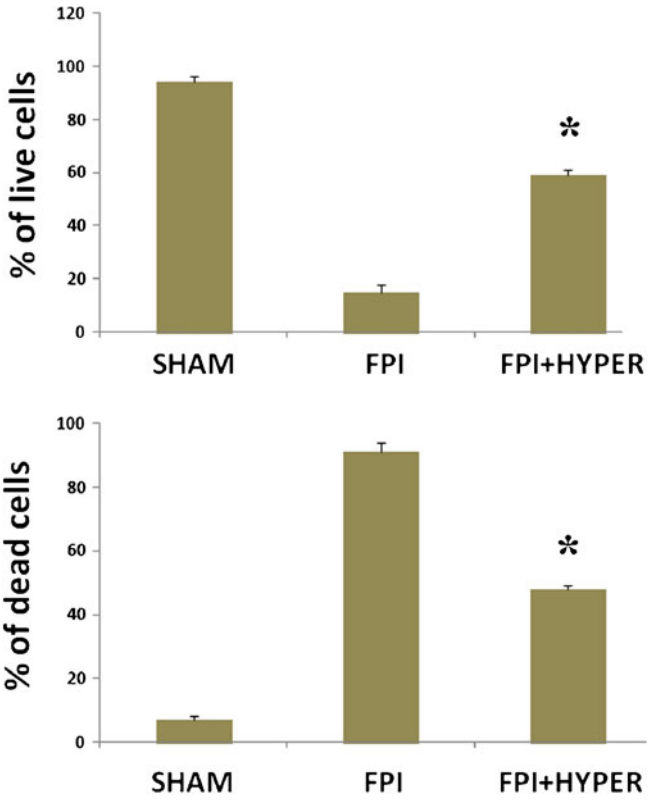

Figure 1. The data include the percentage of live and dead cells from a TUNEL assay following fluid percussion injury and are presented as the mean±SEM (*$p<0.05$ sham vs FPI, FPI vs FPI+Hyperoxia).

3. Results

3.1 *Normabaric hyperoxia on morphological progress*

Figure 2A explains the execution of fluid percussion injury in the right striatum (embedded in paraffin wax) to determine the exact stereotactic implantation of the cannula in the right striatum (figure 2B). There was no significant change ($p<0.05$) in the sham group with regards to morphology, neuronal damage or spiny pyramidal neurons. Figures 3 and 4 illustrate the morphological changes in the right striatum after the induction of fluid percussion injury compared to control. The neurons were shrunken and appeared pyknotic in nature. A few of them also exhibited irregular shapes and tangle-like appearances, while others were large and circular in shape. Treatment with NBO resulted in less neuronal damage in the area of the point of injury. The results have been shown using different magnifications (20× in figure 3 and 40× in figure 4) to facilitate the assessment of the morphological changes at the point of injury.

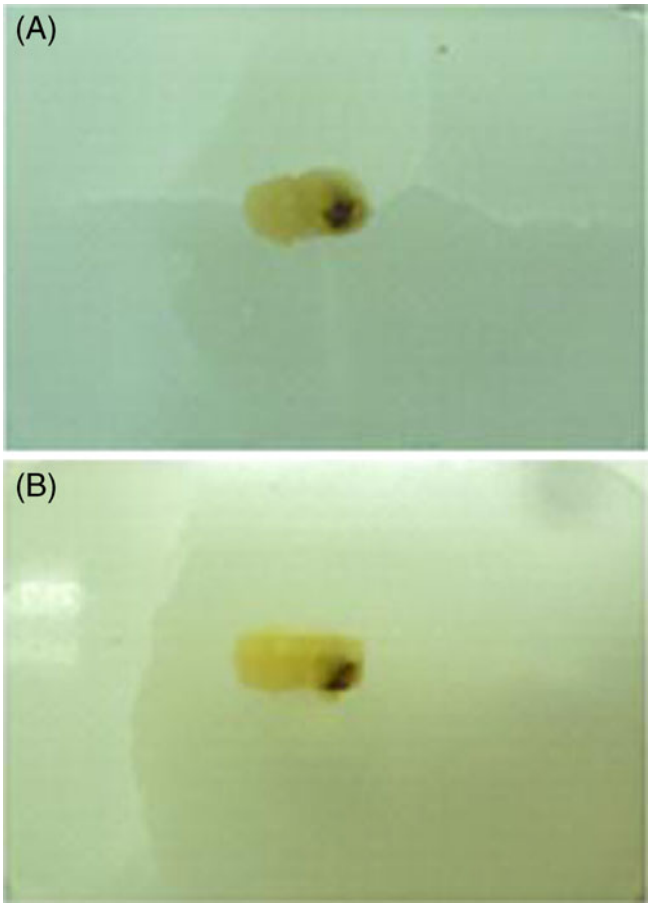

Figure 2. (**A** and **B**) The site of fluid percussion injury in the striatum of mice (sections embedded in wax). The black colour reveals that point of injury.

3.2 *Normabaric hyperoxia on apoptotic cell death*

Figures 5 and 6 demonstrate the degree of apoptotic cell death following the induction of fluid percussion injury on the right striatum of mice after 3 h. The FPI group exhibited significant cell death compared to the control group, whereas the NBO group showed less neuronal damage compared to the TBI group. The morphological characteristics of apoptotic cell death seem to include nuclear and cytoplasmic condensation and the presence of apoptotic bodies. The dead and alive cells were counted manually. There was a significant ($p<0.05$) reduction in cell death in NBO-exposed mice compared to mice subjected to FPI alone.

3.3 *Normabaric hyperoxia on neuronal structure*

Figure 7 revealed structural changes in the soma, axon and spine after the induction of FPI. The FPI group presents with destruction of the neuronal structure within 3 h after the injury, which is in contrast to the control group. Structural changes in the neurons included shorter axonal lengths, irregular shapes of the soma and dislocated spines. Immediate exposure to NBO resulted in less structural damage compared to the TBI group ($p<0.05$).

3.4 *Normabaric hyperoxia on Secondary damage*

Figure 8 demonstrated that immediate exposure to NBO could prevent secondary damage. Figure 8 shows more primary and secondary neuronal damage at the point of injury and in the adjacent area in the FPI group compared to the control group, whereas the FPI group treated with NBO for 3 h immediately after injury presented with less ($p<0.05$) secondary neuronal damage. Figure 8 is a picture of the injury site showing the morphology, neuronal damage and neuronal structure. It confirms the reduction in cell death due to secondary damage in the right striatum after 3 h of NBO exposure (figure 9).

4. Discussion

TBI accounts for up to 80% of clinical TBI cases and can cause neuronal damage that might develop and persist over several years (Tran *et al.* 2006). Pathological examinations of humans and animal models after brain injury reveal damage that might contribute to cognitive and locomotor impairments (Spain *et al.* 2010). Clinically relevant models of TBI

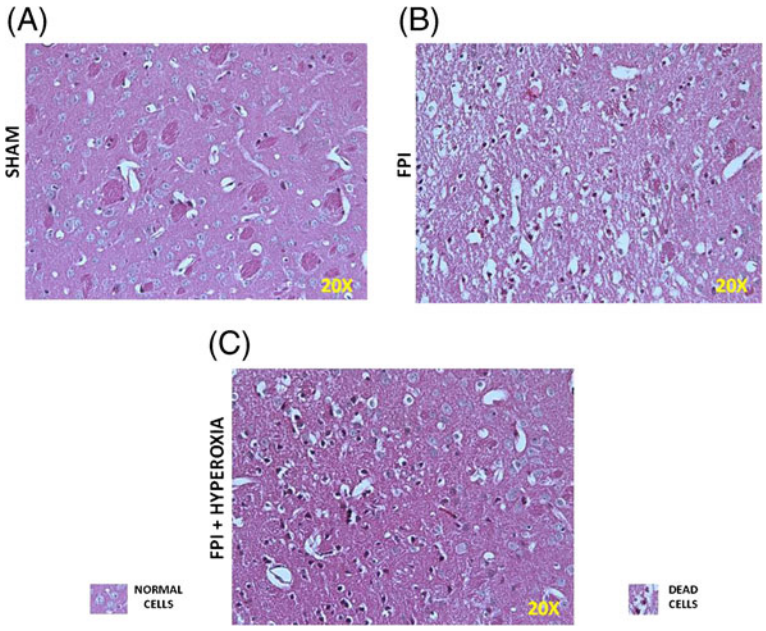

Figure 3. Histological staining (H&E) revealed the morphological changes at the point of injury (20× magnification). Irregular-shaped neurons were considered dead cells. Live cells are circular shaped. (**A**) Sham, (**B**) FPI and (**C**) FPI+Hyperoxia.

for the investigation of neurobiological changes and the development of therapeutic strategies are poorly developed. FPI is a well-characterized experimental model of TBI in mice. Therefore, FPI applied to mice may be a useful experimental tool to investigate TBI at the morphological level. In the present study, we aimed to investigate the effect of immediate exposure to NBO following FPI on the right striatum of mice. The effects of NBO on morphological improvement and neuroprotection have been confirmed using this model.

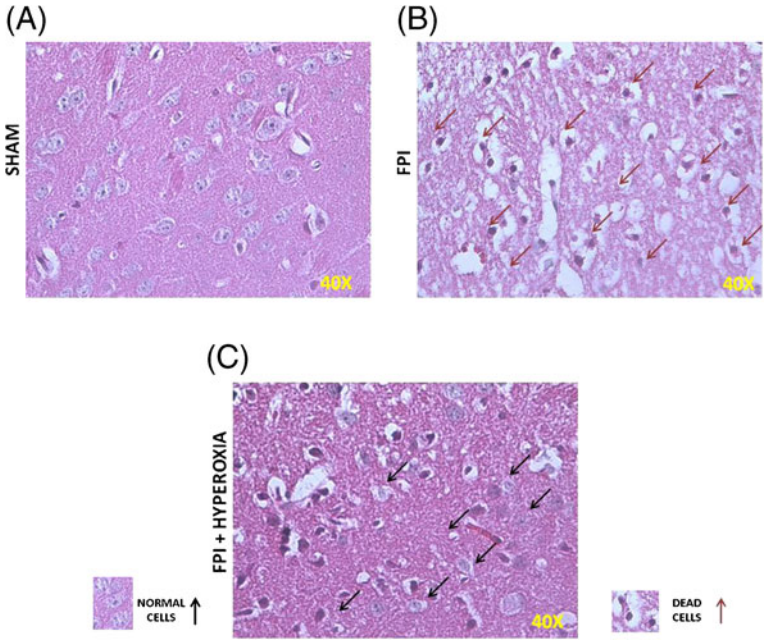

Figure 4. Histological staining (H&E) revealed the morphological changes in the striatum (40× magnification). The alive and dead cells are indicated by black and red arrows, respectively. (**A**) Sham, (**B**) FPI and (**C**) FPI+Hyperoxia.

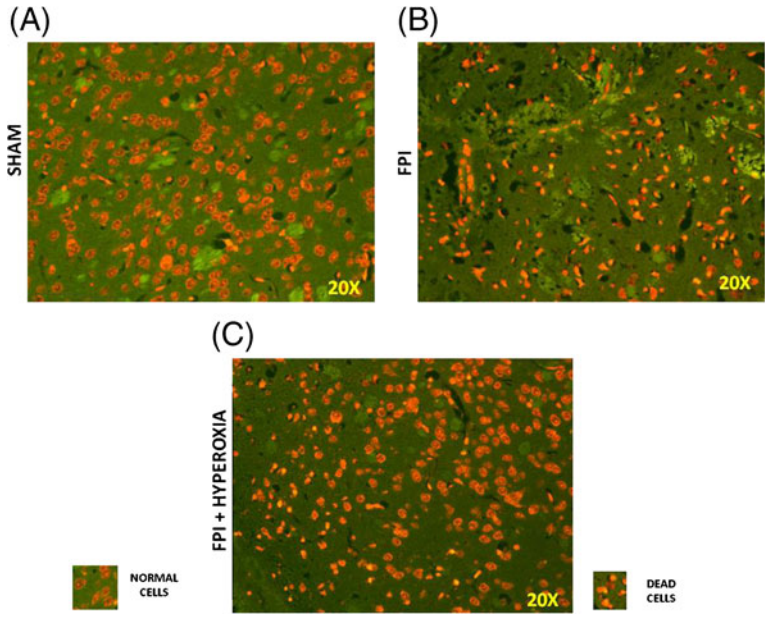

Figure 5. The TUNEL assay demonstrated apoptotic neuronal cell death in the striatum following fluid percussion injury (20× magnification). Yellow-coloured cells were considered dead cell. (**A**) Sham, (**B**) FPI and (**C**) FPI+Hyperoxia.

FPI resulted in morphological changes in the right striatum within 3 h of the injury. The pictures of brain sections embedded in paraffin wax confirmed the site of the injury in the right striatum (figure 2A). There were no morphological changes, and the neuronal damage found in the sham group was identified by morphological, TUNEL and pan-neuronal markers relative to the FPI and FPI+hyperoxia groups (figure 2B). The results in figures 3 and 4 show the morphological changes in the area of the point of injury 20× and 40× magnifications. The purpose of presenting the results at different magnifications is to facilitate the understanding of the damage in the area of

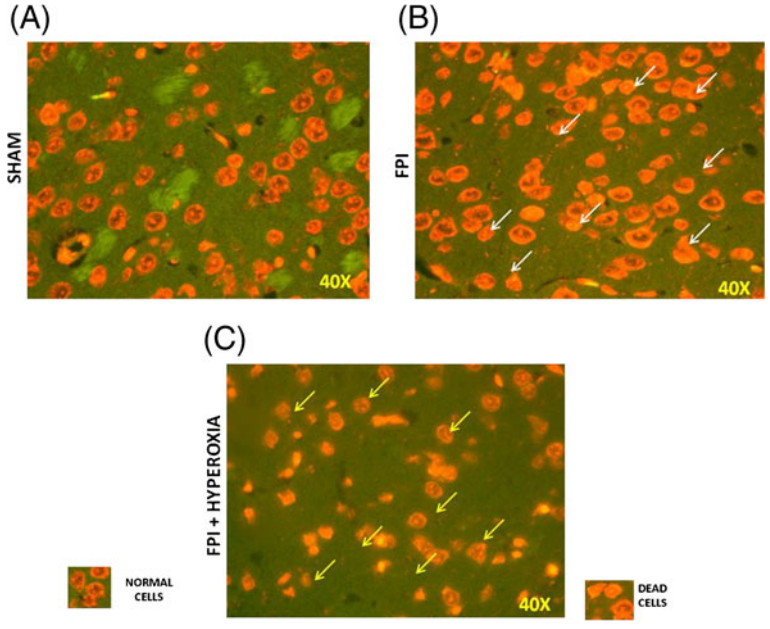

Figure 6. The TUNEL assay revealed neuronal cell death in the striatum (40× magnification). The alive and dead cells are indicated by yellow and white arrows, respectively. (**A**) Sham, (**B**) FPI and (**C**) FPI+Hyperoxia.

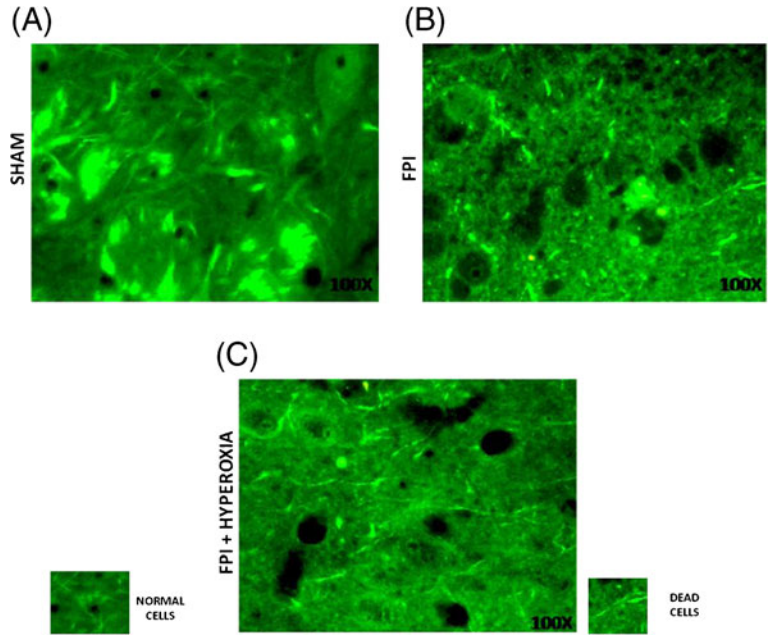

Figure 7. The damage to the complete neuronal structure in the soma, axonal length and spiny pyramidal neurons 3 h after fluid percussion injury in the striatum was detected using pan-neuronal markers. (**A**) Sham, (**B**) FPI and (**C**) FPI+Hyperoxia.

the injury site as well as at the site of the injury. The pictures display the apparent neuronal damage indicted by pyknotic cells, nucleus shrinkage and irregular shapes in the FPI group, whereas the control group did not show any

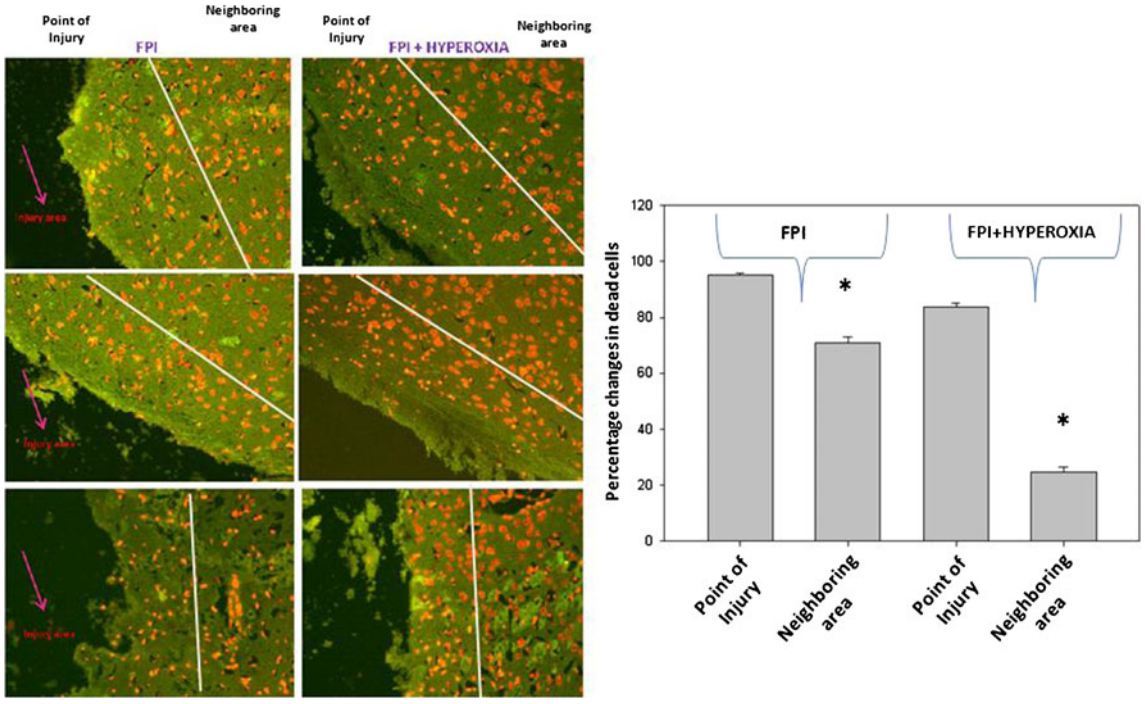

Figure 8. The pictures revealing various points of injury and neighbouring injury sites. These pictures confirm the prevention of secondary neuronal damage by exposure to NBO for 3 h. The data are presented as the mean percentage changes±SEM (*$p<0.05$ sham vs FPI, FPI vs FPI+Hyperoxia).

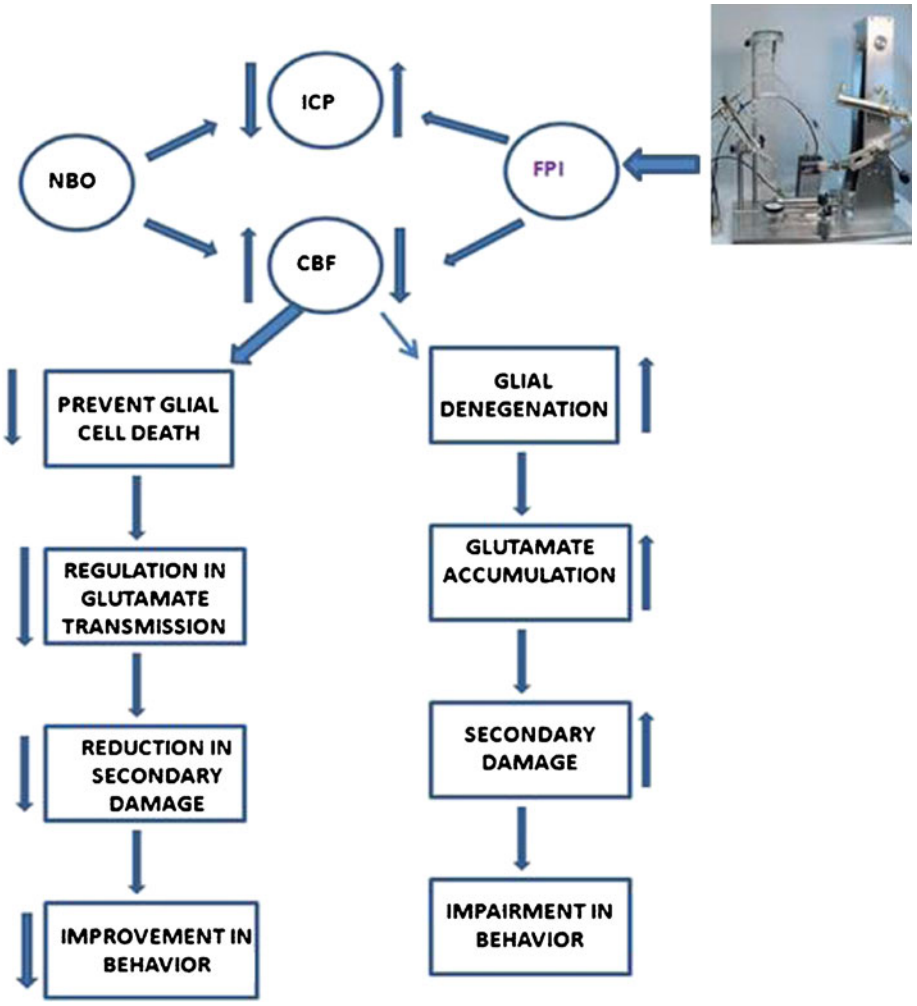

Figure 9. The mechanisms of normabaric hyperoxia exposure prevents secondary damage.

morphological changes in the right striatum. Immediate exposure to normabaric hyperoxia following fluid percussion injury resulted in fewer morphological changes compared to no hyperoxia treatment.

The use of an FPD to induce traumatic brain injury in mice has been reported in previous reports (Erb *et al.* 1988). Additionally, FPI is one of the most frequently used direct brain deformation models. Indeed, it has been found suitable for the study of injury pathology (Conti *et al.* 1998) in a wide range of species, including mice. FPI-induced morphological changes occur due to elevated craniocerebral pressure (Pfenninger *et al.* 1989), decreased cerebral perfusion pressure (Pfenninger *et al.* 1989), reduced cerebral blood flow (Pfenninger *et al.* 1989) and increased cerebral vascular resistance (Pfenninger *et al.* 1989). In addition, alterations in cerebral blood flow (Qian *et al.* 1996) and increased permeability of the blood brain barrier have been demonstrated in FPI models. The most frequently reported histopathological findings after FPI are axonal damage and tissue tears because of cell death or cell loss as well as characteristic vascular damage in the grey/white maters (Graham *et al.* 2000).

Immediate exposure to NBO following FPI could minimise morphological changes due to the increased availability of oxygen for striatal neurons. TBI primary injury, which elevates the brain ICP and disrupts the BBB, may lead to hypoxic-ischemic injury as a result of inadequate oxygen supply to the brain tissues. As a result, brain tissue oxygen tensions (PtiO2) may be increased. NBO treatment is one of the available therapeutic options to decrease ICP and reduce $PtiO_2$. NBO treatment is administered by increasing the inspired oxygen concentration to 100% at normal atmospheric pressure. This treatment is believed to improve brain oxygen metabolism and reduce tissue damage. A human study conducted by Reinert *et al.* indicates that an increased oxygen concentration will

influence brain metabolism, as shown by the reduction in brain lactate accumulation, the reestablishment of the blood brain barrier, the increase in glucose in cellular tissues and the increase in neuronal self-repair.

Following the morphological study, we looked for neuronal damage in the right striatum of mice 3 h after the fluid percussion injury. An FITC-Annexin V apoptosis kit was used to understand the mode of neuronal damage in the striatum. Our results are shown in figures 5 and 6. There was significant neuronal apoptotic cell death after FPI compared to that observed in the control group 3 h post injury. Apoptotic cell death seems to be characterised by nuclear and cytoplasmic condensation and the presence of apoptotic bodies. The results were presented at different magnifications to observe cell death at the exact site of injury (primary damage) as well as at sites proximal to the injury (secondary damage). FPI may cause apoptotic neuronal cell death 3 h post injury in the striatum of mice. Sinson *et al.* (1997) reported that apoptotic cells were identified as early as 24 h after injury induced by the use of a fluid percussion device in rats. Many previous findings suggested that the number of apoptotic cells significantly increased as early as 12 h after injury (Luo *et al.* 2002; Raghupathi *et al.* 2002). Apoptotic cell death mechanisms have been implicated in the pathogenesis of traumatic brain injury (Clark *et al.* 2000; Eldadah *et al.* 2000). FPI-induced apoptotic cell death may be a result of oxidative stress. The brain is vulnerable to oxidative stress due to its high rate of oxidative metabolic activity (Maier *et al.* 2002). Oxidative stress leading to calcium accumulation, mitochondrial dysfunction and the production of reactive oxygen radicals is an important mechanism of cell death following traumatic insults (Lewén *et al.* 2001). After trauma, evidence for the generation of reactive oxygen species (ROS) has been demonstrated in a variety of injury models (Kim *et al.* 2002). Although our data provide evidence that apoptotic cell death may, in part, contribute to striatal cell death in experimental models of traumatic brain injury, the mechanisms underlying apoptotic cell death in the brain following TBI are yet to be determined. Exposure to normabaric hyperoxia for 3 h immediately after fluid percussion injury could significantly reduce apoptotic cell death in the striatum of mice (figure 1B).

For further confirmation of complete neuronal structural damage, we endeavoured to study the complete neuronal structure using the pan-neuronal marker Alexa 488. The result is shown in figure 4, which reveals that 3 h after fluid percussion injury, the neuronal structures of the soma, axon and spine have been destroyed, whereas these in the control group have not been destroyed. Neuronal structural changes caused by FPI include a shorter axonal length, irregular shapes of the soma and dislocated spines. Normabaric hyperoxia could possibly prevent neuronal damage within 3 h of fluid percussion injury.

However, histological damage after TBI can occur far from the point of injury (Gennarelli *et al.* 1998). The primary insult initiates a wide range of secondary injury mechanisms that critically participate in the pathogenesis of TBI. Therefore, in our present study, we also show the secondary damage within 3 h of FPI. Figure 4 demonstrates that exposure to normabaric hyperoxia could reduce secondary damage within 3 h post-injury. The results displayed in figure 3 and 4 suggest that normabaric hyperoxia could minimize the neuronal damage as shown by histology, TUNEL and pan-neuronal markers. Fluid percussion brain injury provides an excellent model for this type of secondary damage resulting from generalized injury; moderate fluid percussion causes very little immediate damage to the brain tissue, but animals are left with pathologically altered neuronal cells and lasting neurological deficits (McIntosh *et al.* 1987).

The severity of the secondary mechanisms of TBI depends upon the severity of the injury (mild, moderate or severe) and the location of the primary insult. In conditions of severe TBI, reductions in CBF have been reported to reach ischemic levels (Zauner *et al.* 1996). Thus, cerebral ischemia is discussed as a secondary injury mechanism that may participate in brain trauma (Marion *et al.* 1991). Although the initial injury often involves irreversible destruction of the tissue, secondary injury processes that are potentially preventable occur in the days and weeks after injury. This secondary damage causes pathological changes in cells over a much larger area than the primary injury and is often responsible for many of the lasting neurological deficits associated with TBI (Siesjö *et al.* 1996). Central fluid percussion, in particular, is associated with reduced cell death when compared with lateral fluid percussion.

The reduction in secondary neuronal damage after hyperoxia treatment may be due to improvements in the oxidative metabolism of the brain (Tolias *et al.* 2004). The cerebrovascular response to hyperoxia has been investigated in physiological and pathological conditions, and it has been shown that there is a cerebral blood flow (CBF) reduction in response to hyperoxia ranging from 9% to 27% (Johnston *et al.* 2003). In addition, Rockswold *et al.* (2001) investigated the effects of hyperoxia therapy at 1.5 atm on CBF and metabolism. The authors observed that at 1 and 6 h post treatment, there was an increase in CBF in the group with decreased baseline values, whereas there was a decrease in CBF in the patients with increased pretreatment values. Patients with CBF within the normal range showed similar values before and after exposure to hyperoxia. Focal areas of reduced CBF after brain trauma can be surrounded by regions exhibiting milder reductions in

flow (Dietrich *et al.* 1996). This surrounding area may thus correspond to the penumbral region surrounding an ischemic core (Hossmann *et al.* 1994). This border zone area contains scattered damaged neurons within an intact neuropil (Dietrich *et al.* 1994). Importantly, this area is sensitive to therapeutic interventions and is at risk for secondary insults (Bramlett *et al.* 1999). Thus, the risk of secondary neuronal damage should be reduced by therapeutic interventions, such as hyperoxia treatment. The increased level of CBF could be one of the reasons for the minimised secondary damage when hyperoxia is used as a treatment after traumatic brain injury. Apart from hyperoxia treatment, various pathways could be investigated to develop methods to reduce secondary damage. Many of these pathways could be associated with calcium and calcium-modulated systems (Tymianski *et al.* 1996). Additionally, oxidative stress may contribute to many of the pathophysiological changes that occur after TBI (Tyurin *et al.* 2000) and may be responsible for glutamate dysregulation in the post-traumatic period (Hinzman *et al.* 2010). Normabaric hyperoxia treatment could control the oxidative stress- and calcium-induced neuronal damage through glutamate excitotoxicity in the striatum following fluid percussion injury. These mechanisms could be involved in reducing the secondary neuronal damage after exposure to normabaric hyperoxia treatment within 3 h of the fluid percussion injury.

5. Conclusion

FPI is a well-established model for inducing traumatic brain injury. TBI causes secondary neuronal damage over time. After injury, cells have an inadequate oxygen supply but need oxygen to recover from secondary damage. Immediately following TBI, ICP increased and CBF decreased, which could be the reason for this further damage. Based on the literature and our present results, immediate exposure to NBO could serve as a possible therapeutic protocol to minimize the secondary neuronal damage by reducing ICP and increasing CBF. We suggest that immediate exposure to NBO could be a useful treatment for victims of TBI.

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References

Bramlett HM, Green EJ, Dietrich WD, *et al.* 1999 Exacerbation of cortical and hippocampal CA1 damage due to posttraumatic hypoxia following moderate fluid-percussion brain injury in rats. *J. Neurosurg*. **91** 653–659

Carbonell WS, Maris DO, McCall T, Grady MS, *et al.* 1998 Adaptation of the fluid percussion injury model to the mouse. *J. Neurotrauma* **15** 217–229

Chopp M, Li Y, Dereski MO, Levine SR, Yoshida Y, Garcia JH, *et al.* 1991 Neuronal injury and expression of 72-kDa heat-shock protein after forebrain ischemia in the rat. *Acta Neuropathol*. **83** 66–71

Clark RS, Kochanek PM, Watkins SC, Chen M, Dixon CE, Seidberg NA, Melick J, Loeffert JE, Nathaniel PD, Jin KL, Graham SH, *et al.* 2000 Caspase-3 mediated neuronal death after traumatic brain injury in rats. *J. Neurochem*. 74 740–753

Conti AC, Raghupathi R, Trojanowski JQ, McIntosh TK, *et al.* 1998 Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. *J. Neurosci*. **18** 5663–5672

Dietrich WD, Alonso O, Busto R, Prado R, Dewanjee S, Dewanjee MK, Ginsberg MD, *et al.* 1996 Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: a double-label autoradiographic study in rats. *J. Cereb. Blood Flow Metab.* **16** 481–489

Dietrich WD, Alonso O, Halley M, *et al.* 1994 Early microvascular and neuronal consequences of traumatic brain injury: a light and electron microscopic study in rats. *J. Neurotrauma* 11 289–301

El Massioui N, Chéruel F, Faure A, Conde F, *et al.* 2007 Learning and memory dissociation in rats with lesions to the subthalamic nucleus or to the dorsal striatum. *Neuroscience* **147** 906–918

Eldadah BA, Faden AI, *et al.* 2000 Caspase pathways, neuronal apoptosis, and CNS injury. *J. Neurotrauma* **17** 811–829

Erb DE, Povlishock JT, *et al.* 1988 Axonal damage in severe traumatic brain injury: an experimental study in cat. *Acta Neuropathol*. **76** 347–358

Gennarelli TA, Graham DI, *et al.* 1998 Neuropathology of the head injuries. *Semin. Clin. Neuropsychiatry* **3** 160–175

Graham DI, Raghupathi R, Saatman KE, Meaney D, McIntosh TK, *et al.* 2000 Tissue tears in the white matter after lateral fluid percussion brain injury in the rat: relevance to human brain injury. *Acta Neuropathol*. **99** 117–124

Haruddin FA 2010 Normobaric hyperoxia treatment in traumatic brain injury: a focus on basal ganglia. *Orient. Neuron Nexus* **1** 13–16

Hinzman JM, Thomas TC, Burmeister JJ, Quintero JE, Huettl P, Pomerleau F, Gerhardt GA, Lifshitz J, *et al.* 2010 Diffuse brain injury elevates tonic glutamate levels and potassium-evoked glutamate release in discrete brain regions at two days post-injury: an enzyme-based microelectrode array study. *J. Neurotrauma* **27** 889–899

Hossmann KA, *et al.* 1994 Viability thresholds and the penumbra of focal ischemia. *Annal. Neurol*. **36** 557–565

Johnston AJ, Steiner LA, Gupta AK, Menon DK, *et al.* 2003 Cerebral oxygen vasoreactivity and cerebral tissue oxygen reactivity. *Br. J. Anaesth*. **90** 774–786

Kim GW, Kondo T, Noshita N, Chan PH, *et al.* 2002 Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. *Stroke* **33** 809–815

Lewén A, Fujimura M, Sugawara T, Matz P, Copin JC, Chan PH, *et al.* 2001 Oxidative stress-dependent release of mitochondrial cytochrome c after traumatic brain injury. *J. Cereb. Blood Flow Metab.* **21** 914–920

Luo C, Jiang J, Lu Y, Zhu C, *et al.* 2002 Spatial and temporal profile of apoptosis following lateral fluid percussion brain injury. *Chin. J. Traumatol*. **5** 24–27

Maier CM, Chan PH, *et al.* 2002 Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. *Neuroscientist* **8** 323–334

Marion DW, Darby J and Yonas H 1991 Acute regional cerebral blood flow changes caused by severe head injuries. *J. Neurosurg*. **74** 407–414

McIntosh TK, Noble L, Andrews B, Faden AI, *et al.* 1987 Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. *Cent. Nerv. Syst. Trauma* **4** 119–134

Muthuraju S, Pati S, Rafiqul M, Abdullah J and Jaafar H 2012 IntelliCage provides voluntary exercise and an enriched environment, improving locomotive activity in mice following fluid percussion injury. *Basal Ganglia* **2** 143–151

Pfenninger EG, Reith A, Breitig D, Grünert A, Ahnefeld FW, *et al.* 1989 Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. Part 1: An experimental study of the underlying pathophysiology. *J. Neurosurg*. **70** 774–779

Qian L, Ohno K, Maehara T, Tominaga B, Hirakawa K, Kuroiwa T, Takakuda K, Miyairi H, *et al.* 1996 Changes in lCBF, morphology and related parameters by fluid percussion injury. *Acta Neurochir.* **138** 90–98

Raghupathi R, Conti AC, Graham DI, Krajewski S, Reed JC, Grady MS, Trojanowski JQ, McIntosh TK, *et al.* 2002 Mild traumatic brain injury induces apoptotic cell death in the cortex that is preceded by decreases in cellular Bcl-2 immunoreactivity. *Neuroscience* **110** 605–616

Rockswold SB, Rockswold GL, Vargo JM, Erickson CA, Sutton RL, Bergman TA, Biros MH, *et al.* 2001 Effects of hyperbaric oxygenation therapy on cerebral metabolism and intracranial pressure in severely brain injured patients. *J. Neurosurg*. **94** 403–411

Siesjö BK, Siesjö P, *et al.* 1996 Mechanisms of secondary brain injury. *Eur. J. Anaesthesiol*. **13** 247–268

Sinson G, Perri BR, Trojanowski JQ, Flamm ES, McIntosh TK, *et al.* 1997 Improvement of cognitive deficits and decreased cholinergic neuronal cell loss and apoptotic cell death following neurotrophin infusion after experimental traumatic brain injury. *J. Neurosurg*. **86** 511–518

Spain A, Daumas S, Lifshitz J, Rhodes J, Andrews PJ, Horsburgh K, Fowler JH, *et al.* 2010 Mild fluid percussion injury in mice produces evolving selective axonal pathology and cognitive deficits relevant to human brain injury. *J. Neurotrauma* **27** 1429–1438

Tolias CM, Reinert M, Seiler R, Gilman C, Scharf A, Bullock MR, *et al.* 2004 Normobaric hyperoxia—induced improvement in cerebral metabolism and reduction in intracranial pressure in patients with severe head injury: a prospective historical cohort-matched study. *J. Neurosurg.* **101** 435–444

Tran LD, Lifshitz J, Witgen BM, Schwarzbach E, Cohen AS, Grady MS, *et al.* 2006 Response of the contralateral hippocampus to lateral fluid percussion brain injury. *J. Neurotrauma* **23** 1330–1342

Tymianski M, Tator CH, *et al.* 1996 Normal and abnormal calcium homeostasis in neurons: a basis for the pathophysiology of traumatic and ischemic central nervous system injury. *Neurosurgery* **38** 1176–1195

Tyurin VA, Tyurina YY, Borisenko GG, Sokolova TV, Ritov VB, Quinn PJ, Rose M, Kochanek P, Graham SH, Kagan VE, *et al.* 2000 Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. *J. Neurochem*. **75** 2178–2189

Voulalas PJ, Holtzclaw L, Wolstenholme J, Russell JT, Hyman SE, *et al.* 2005 Metabotropic glutamate receptors and dopamine receptors cooperate to enhance extracellular signal-regulated kinase phosphorylation in striatal neurons. *J. Neurosci*. **25** 3763–3773

Xu J, Millard M, Ren X, Cox OT, Erdreich-Epstein A, *et al.* 2010 c-Abl mediates endothelial apoptosis induced by inhibition of integrins alphavbeta3 and alphavbeta5 and by disruption of actin. *Blood* **115** 2709–2718

Zauner A, Bullock R, Kuta AJ, Woodward J, Young HF, *et al.* 1996 Glutamate release and cerebral blood flow after severe human head injury. *Acta Neurochir. Suppl*. **67** 40–44

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